

Relationship between the cAMP levels in leukocytes and the cytokine balance in patients surviving gram negative bacterial pneumonia

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Lipopolysaccharide-stimulated leukocytes secrete proinflammatory cytokines including tumor necrosis factor- α and interleukin-12. Over-activation of host defense systems may result in severe tissue damage and requires regulation. Granulocyte colony-stimulating factor and interleukin-10 are candidate cytokines for inducing tolerance to lipopolysaccharide re-stimulation. We compared cytokines secreted by lipopolysaccharide-stimulated blood cells from patients who had survived gram negative bacterial pneumonia (*Pseudomonas aeruginosa*, *Escherichia coli* or *Proteus mirabilis*, $n = 26$) and age-matched healthy volunteers ($n = 18$). Interleukin-12p70 and tumor necrosis factor- α expression was significantly lower in patients ($p = 0.0039$ and $p < 0.001$) compared to healthy controls, while granulocyte colony-stimulating factor production was markedly higher in patients ($p < 0.001$). Levels of interleukin-10 were comparable. Granulocyte colony-stimulating factor expression was inversely correlated with interleukin-12p70 ($R = -0.71$, $p < 0.001$) and tumor necrosis factor- α ($R = -0.64$, $p < 0.001$) expression; interleukin-10 showed no significant correlation. In unstimulated leukocytes from patients, cAMP levels were significantly raised ($p = 0.020$) and were correlated inversely with interleukin-12p70 levels ($R = -0.81$, $p < 0.001$) and directly with granulocyte colony-stimulating factor ($R = 0.72$, $p = 0.0020$), matrix metalloproteinase-9 ($R = 0.67$, $p = 0.0067$) and interleukin-10 ($R = 0.54$, $p = 0.039$) levels. Our results demonstrate that granulocyte colony-stimulating factor production by lipopolysaccharide-stimulated leukocytes is a useful indicator of tolerance induction in surviving pneumonia patients and that measuring cAMP in freshly isolated leukocytes may also be clinically significant.

Key Words: cAMP, granulocyte colony-stimulating factor, tolerance, lipopolysaccharide, interleukin-12

Lipopolysaccharides (LPS) in the outer membrane of gram negative bacteria are endotoxins that elicit strong immune responses and act as well-recognized alarm signals in human hosts.⁽¹⁾ Stimulating leukocytes with LPS *in vitro* induces the secretion of proinflammatory cytokines such as tumor necrosis factor (TNF)- α and interleukin (IL)-12 which kill the bacteria but may also cause severe tissue damage in the host. The regulation of over-activation of this defense system is therefore very important to protect the host, especially when exposed to prolonged or recurrent alarm signals such as LPS.

Critical roles for IL-12 have been identified not only in the T

helper type 1 immune response and host defenses against intracellular microorganisms^(2,3) but also in combating extracellular microorganisms. Yamamoto *et al.* showed that IL-12 plays a critical role in the early phase of bacterial pneumonia by promoting the recruitment of neutrophils to infected lung tissues, where TNF- α production is dependent on IL-12, using IL-12p40-knockout mice.⁽⁴⁾ IL-12 is a chemotactic factor for both neutrophils and NK cells and induces their adherence to vascular endothelial cells.⁽⁵⁾ Preoperative levels of IL-12 secretion by monocytes stimulated *in vitro* with LPS were significantly lower in patients who developed lethal postoperative sepsis, compared with the survivors.⁽⁶⁾ However, IL-12 has been closely associated with lung tissue injury, as the development of bleomycin-induced pneumopathy is prevented by treating normal mice⁽⁷⁾ and IL-12p40-knockout mice with anti-IL-12 antibody.⁽⁸⁾ IL-12 also reduces the expression of matrix metalloproteinase (MMP)-9, which is associated with angiogenesis.⁽⁹⁾

Granulocyte colony-stimulating factor (G-CSF)⁽¹⁰⁾ and IL-10^(11,12) are likely candidates for cytokines that induce tolerance to repeated LPS stimulation. *In vivo* treatment with recombinant G-CSF not only results in the mobilization of hematopoietic precursor cells⁽¹³⁾ and neutrophils⁽¹⁴⁾ but also results in the appearance of immunoregulatory cells, such as type 2 dendritic cells⁽¹⁵⁾ and type 2 helper T cells,⁽¹⁶⁾ in human blood. Pretreatment with G-CSF attenuates the LPS-stimulated secretion of proinflammatory cytokines, such as IL-12 and TNF- α , and simultaneously enhances IL-10 secretion.⁽¹⁷⁾ G-CSF treatment also increases serum protein levels of hepatocyte growth factor⁽¹⁸⁾ and plasma protein levels of MMP-9.⁽¹⁹⁾ In contrast, in neutrophils G-CSF is known to inhibit apoptosis⁽²⁰⁾ and IL-10 reverses the anti-apoptotic effect of LPS.⁽²¹⁾ Taken together, these observations suggest that G-CSF acts not only as an anti-inflammatory cytokine but also as a defense against infections and a promoter of tissue repair.

A response to LPS requires its presentation by CD14 to Toll-like receptor (TLR) 4.⁽²²⁾ Among circulating blood cells, monocytes express relatively high levels of cell surface TLR4 and CD14, while neutrophils also express them both but at low levels. Basophils express TLR4 but not CD14 and eosinophils and most lymphocytes express neither molecule.^(23,24) Monocytes are an important source of both the proinflammatory cytokines IL-12 and

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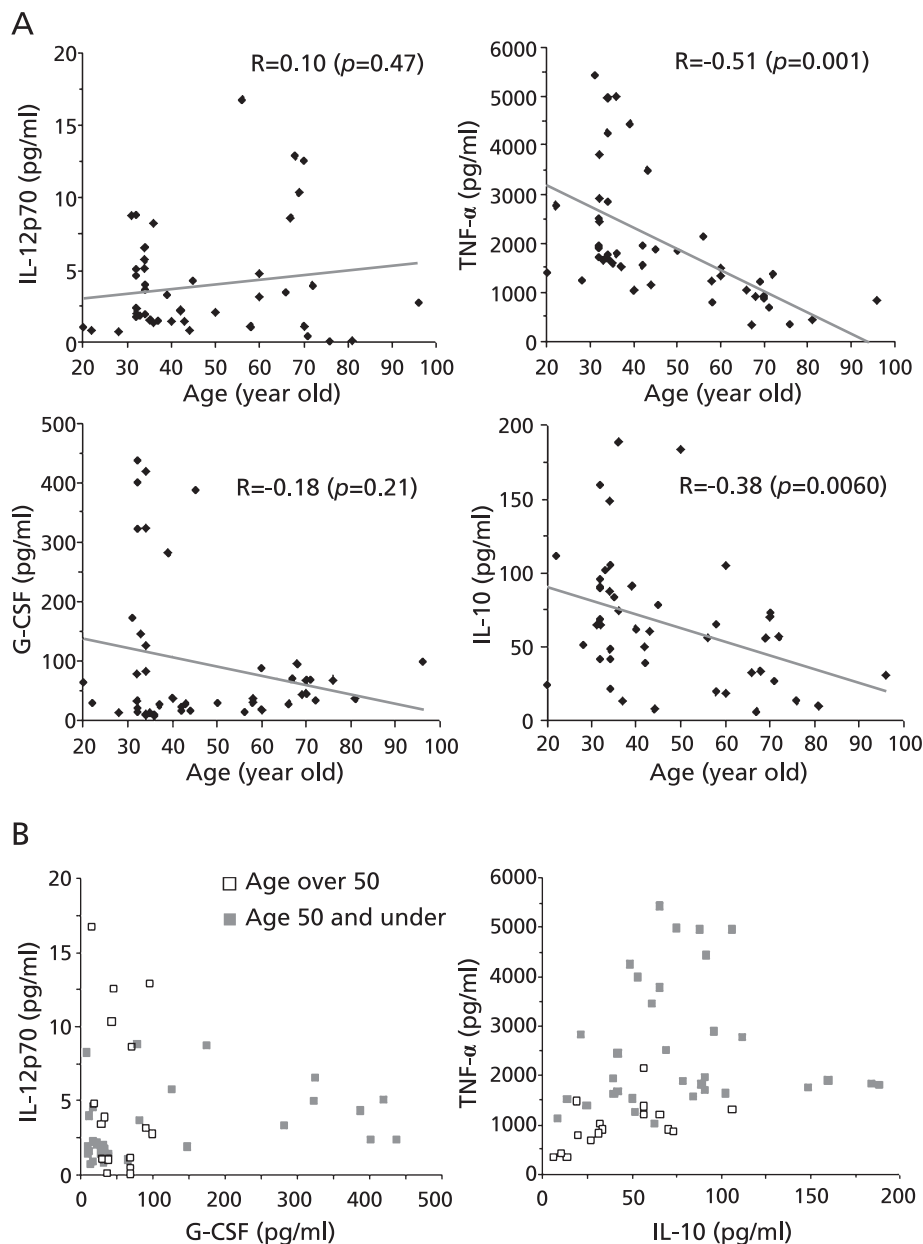


Fig. 1. Effect of aging on cytokine production by LPS-stimulated leukocytes. (A) Blood from healthy volunteers ($n = 47$) ranging from 20 to 96 y of age was incubated in the presence of 10 $\mu\text{g/ml}$ LPS for 24 h and cytokine concentrations in the supernatants were then measured by ELISA. Correlation coefficients (R) were calculated by linear regression analysis. (B) Comparison of data from volunteers aged over 50 y (open squares) and 50 y or under (closed squares).

TNF- α and the anti-inflammatory cytokines G-CSF and IL-10. However, human neutrophils have also been reported to produce physiologically relevant levels of IL-12⁽²⁴⁾ and IL-10.⁽²⁵⁾

In this study, we investigated the production of TNF- α , IL-12, CSF and IL-10 in LPS-tolerant patients who had survived gram negative bacterial pneumonia with no severe inflammatory symptoms. We also measured cyclic adenosine monophosphate (cAMP) in unstimulated leukocytes from these patients to elucidate the relationship between cAMP levels and the profile of cytokines secreted in response to LPS, because recent *in vitro* studies have shown that intracellular cAMP concentrations in monocytes can determine the secretion of these cytokines⁽²⁶⁻²⁸⁾ although LPS itself does not affect cAMP levels.⁽²⁹⁾

Materials and Methods

Patients. We enrolled patients over 50 years of age who had survived aspiration pneumonia due to gram negative rods as *Pseudomonas aeruginosa* (*P. aeruginosa*), *Escherichia coli* (*E. coli*) or *Proteus mirabilis* (*P. mirabilis*). All patients had been diagnosed with gram negative bacterial pneumonia from chest X-rays, computed tomography and sputum cultures. Their informed consent was obtained and blood samples were collected at least one week after withdrawal of antibiotics and/or anti-inflammatory drugs. Control blood samples were collected from healthy volunteers who had experienced no episodes of pneumonia within the last 20 years or any infectious diseases within the last 3 months.

Quantifying cAMP levels in leukocytes. Blood (10 ml)

Table 1. Clinical data for the enrolled patients

Patient	Age	Sex	Bacteria in sputum cultures	Leukocyte (cells/ μ l)	CRP (mg/dl)	TP (g/dl)	DM	CVD
1	54	Female	<i>E. coli</i>	2,600	1.2	7.4	+	+
2	55	Male	<i>P. aeruginosa</i>	8,000	3.3	7.6	+	+
3	67	Male	<i>P. aeruginosa</i>	8,400	5.8	6.7	+	+
4	68	Male	<i>P. aeruginosa</i>	8,000	3.5	6.5	+	+
5	68	Female	<i>P. aeruginosa</i>	5,000	3.1	7.7	+	+
6	70	Male	<i>P. mirabilis</i>	6,300	1.5	6.8	+	+
7	70	Female	<i>P. aeruginosa</i>	5,000	0.7	7.7	-	+
8	72	Male	<i>P. aeruginosa</i>	6,500	1.4	7.7	+	+
9	72	Male	<i>P. aeruginosa</i>	6,900	1	7.1	+	+
10	74	Male	<i>P. aeruginosa</i>	9,800	4.2	6.5	-	+
11	74	Female	<i>P. aeruginosa</i>	4,000	1	6.8	-	-
12	74	Female	<i>P. aeruginosa</i>	8,500	1.5	6.8	+	+
13	77	Female	<i>P. mirabilis</i>	7,400	2.3	6.7	+	+
14	79	Male	<i>E. coli</i>	7,400	3.7	7.1	-	-
15	79	Female	<i>P. mirabilis</i> , MRSA	4,300	4.3	5.9	-	+
16	82	Female	<i>P. aeruginosa</i>	8,700	4.1	6.6	-	+
17	82	Female	<i>P. aeruginosa</i>	4,500	0.8	6.9	-	+
18	84	Male	<i>P. aeruginosa</i> , MRSA	11,000	3.9	7.1	-	+
19	88	Male	<i>P. aeruginosa</i>	9,100	1.9	7.1	+	+
20	88	Male	<i>P. aeruginosa</i>	7,600	2.8	7	+	-
21	90	Female	<i>E. coli</i>	8,700	1.5	5.7	-	+
22	92	Male	<i>E. coli</i> , MRSA	7,900	4.7	6.8	-	+
23	93	Female	<i>P. aeruginosa</i>	8,400	1.1	6.3	+	+
24	94	Female	<i>P. mirabilis</i>	5,200	3.4	7.1	-	-
25	94	Female	<i>E. coli</i>	6,500	1.2	6.3	-	+
26	95	Female	<i>P. aeruginosa</i>	3,300	4.1	6.1	-	+

was collected using a syringe containing 0.5 ml of heparin (Mochida Pharmaceutical, Tokyo, Japan), mixed with 5 ml 6% dextran and incubated for 30 min at room temperature. The enriched leukocyte fraction was collected and centrifuged at 400 g for 5 min, and any residual erythrocytes were then lysed by adding 3 ml ACK lysing buffer (BioWhittaker, Walkersville, MD) for 7 min at room temperature. After washing three times with PBS, the leukocytes were adjusted to 1×10^6 cells/ml. 200 μ l aliquots were lysed with ACK lysing buffer according to the manufacturer's instructions and used to measure cAMP concentrations with an enzyme-linked immunosorbent assay (ELISA) kit (RPN225, GE Healthcare, Buckinghamshire, UK).

Cell culture. LPS-stimulated supernatants were obtained from 40 μ l aliquots of heparinized whole blood, which were mixed with 160 μ l DMEM (Life Technologies, Rockville, MD) in the presence of 10 μ g/ml LPS (Sigma, St. Louis, MO), in triplicate in flat bottomed 96-well microplates (Becton Dickinson Labware, NJ). After incubation for 24 h at 37°C in humidified air containing 5% CO₂, the cells were centrifuged at 400 g for 5 min and the supernatants were collected and stored at -80°C until their use in assays.

Quantifying cytokines secreted by LPS-stimulated leukocytes. The concentrations of IL-12p70 (the active heterodimer of IL-12p40 and p35), TNF- α , G-CSF, IL-10 and MMP-9 in the culture supernatants were quantified using commercial ELISA kits (Biosource International, CA).

Statistical analysis. Data were imported into Statview 5.0 (SAS Institute, Cary, NC) and plotted using log-log, semi-log or linear coordinates. Regression analyses were performed to assess the correlation coefficients (R) and Fisher's protected least significant difference was used to evaluate the degree of fit of the data to power-law distributions. The Mann-Whitney *U* test was used to determine the statistical significance of differences between two experimental groups.

Results

Effect of aging on the production of pro-inflammatory and anti-inflammatory cytokines. Blood from 47 healthy volunteers between 20 and 96 years of age were stimulated *in vitro* with LPS and the cytokines secreted were measured. Fig. 1A shows the relationship between the age of the volunteers and the concentration of the pro-inflammatory and anti-inflammatory cytokines secreted. TNF- α production was inversely correlated with age ($R = -0.51$, $p < 0.001$). IL-10 secretion also dropped with age, but with a lower correlation ($R = -0.38$, $p = 0.0060$). In contrast, production of IL-12p70 and G-CSF was not significantly correlated with age, although amongst individuals aged over 50 there were no outliers showing high production of these cytokines. The plots shown in Fig. 1B compare cytokine production in volunteers over 50 (open squares) with those 50 years or younger (closed squares). Interestingly, no older volunteers showed extraordinarily high levels of production of any cytokine, compared to younger volunteers, and there were no individuals who showed exceptionally high levels of both IL-12p70 and G-CSF production in response to LPS.

Cytokine production in patients surviving gram negative bacterial pneumonia. Table 1 shows the clinical data for the patients enrolled in this study. The 26 patients were aged between 54 and 95 years with a median age of 78 years. Sputum cultures identified *P. aeruginosa* in 17 patients, *E. coli* in 5 patients, and *P. mirabilis* in 4 patients. Methicillin-resistant *Staphylococcus aureus* (MRSA) was also detected in 3 patients. The mean CRP level was 2.6 ± 1.5 mg/dl and the mean leukocyte count in freshly drawn blood was $6,885 \pm 2,109$ cells/ μ l. The proportion of neutrophils and monocytes was more than 50% of total leukocytes in all patients. Percutaneous endoscopic gastrostomy tube (PEG) was placed into 19 patient's stomach and the mean level of total protein (TP) of all the patients was 6.8 ± 0.5 g/dl.

Fig. 2A shows a comparison of the cytokines secreted by LPS-

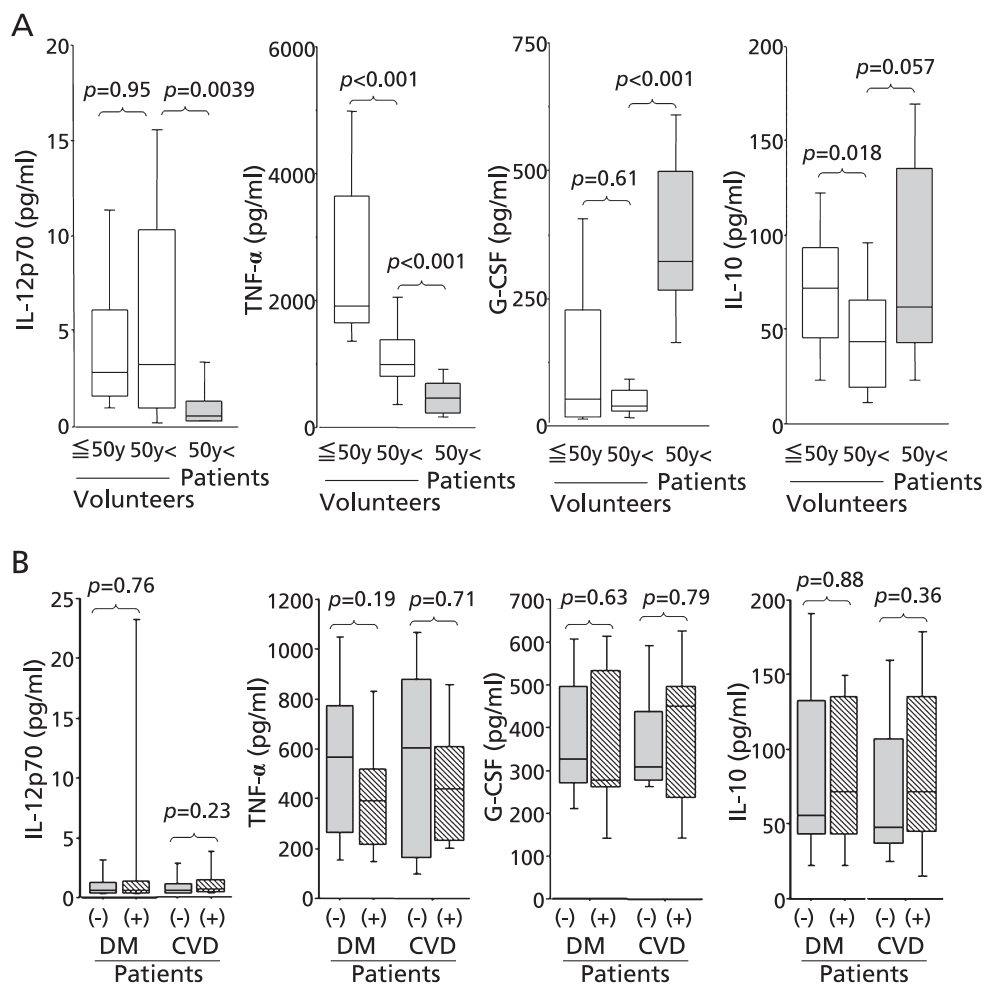


Fig. 2. Changes in cytokine production in patients with gram negative bacterial pneumonia. (A) Blood from volunteers aged 50 years and under ($n = 29$), volunteers aged over 50 years ($n = 18$) and patients with gram negative bacterial pneumonia ($n = 26$) was incubated in the presence of $10 \mu\text{g/ml}$ LPS for 24 h and cytokine concentrations in the supernatants were compared statistically between different pairs of groups. (B) Comparison of cytokine production in patients with or without the additional complications of diabetes mellitus (DM) and cerebrovascular disease (CVD).

stimulated blood from patients ($n = 26$), age-matched healthy volunteers over 50 years old ($n = 18$) and healthy volunteers who were 50 y old or younger ($n = 29$). In the patient group, production of IL-12p70 was significantly lower ($p = 0.0039$) than in age-matched healthy volunteers. TNF- α production was also lower in the older, compared to younger, volunteer groups and lower still in the patient group ($p < 0.001$). G-CSF production was markedly higher in patients compared to both healthy volunteer groups ($p < 0.001$), while IL-10 production was similar in all three groups. Amongst the patient group, 13 people suffered from diabetes mellitus (DM) and 22 people suffered from cerebrovascular disease (CVD). However, these possible confounding factors did not significantly affect the cytokine production in these patients, as shown in Fig. 2B.

Correlation between production of pro-inflammatory and anti-inflammatory cytokines. When we analyzed the production of cytokines, we found that the correlation coefficients between any pair of cytokines we measured were higher in log-log (power-law) distributions than in semi-log or linear distributions. Fig. 3 shows curves modeled for the production of cytokine X and cytokine Y using a power-law distribution in which Y is proportional to X^a , where a is the exponent of the power law. The production of IL-12p70 and TNF- α showed a strong direct correlation ($R = 0.57$, $p < 0.001$, $a = 0.39$). The production of G-

CSF was inversely correlated with the production of both IL-12p70 ($R = -0.71$, $p < 0.001$, $a = -0.74$) and also TNF- α ($R = -0.64$, $p < 0.001$, $a = -0.45$). These results cannot be fully explained by secondary effects of secreted G-CSF, by an autocrine or paracrine mechanism, because we found that the addition of even a high concentration of recombinant G-CSF to the cultures reduced the secretion of IL-12 by at most 34% and TNF- α by at most 28%. The degree of correlation between G-CSF and IL-10 production was lower ($R = 0.35$, $p = 0.020$, $a = 0.38$) and IL-10 production showed no significant correlation with either IL-12p70 or TNF- α production.

Correlation between cytokine and MMP-9 production. Secretion of MMP-9 by blood cells stimulated with LPS *in vitro* was significantly higher in the patient group compared to healthy volunteers ($p = 0.011$; Fig. 4A). As shown in Fig. 4B, MMP-9 production was very closely correlated with G-CSF ($R = 0.64$, $p < 0.001$, $a = 0.36$) and IL-10 ($R = 0.35$, $p = 0.020$, $a = 0.26$) production. Conversely, MMP-9 levels were inversely correlated with TNF- α levels ($R = -0.41$, $p = 0.0047$, $a = -0.34$), but there was no significant correlation between MMP-9 and IL-12p70 production.

Correlation between cAMP levels in leukocytes and cytokine production. We next quantified and analyzed the cAMP content of freshly isolated leukocytes. As shown in

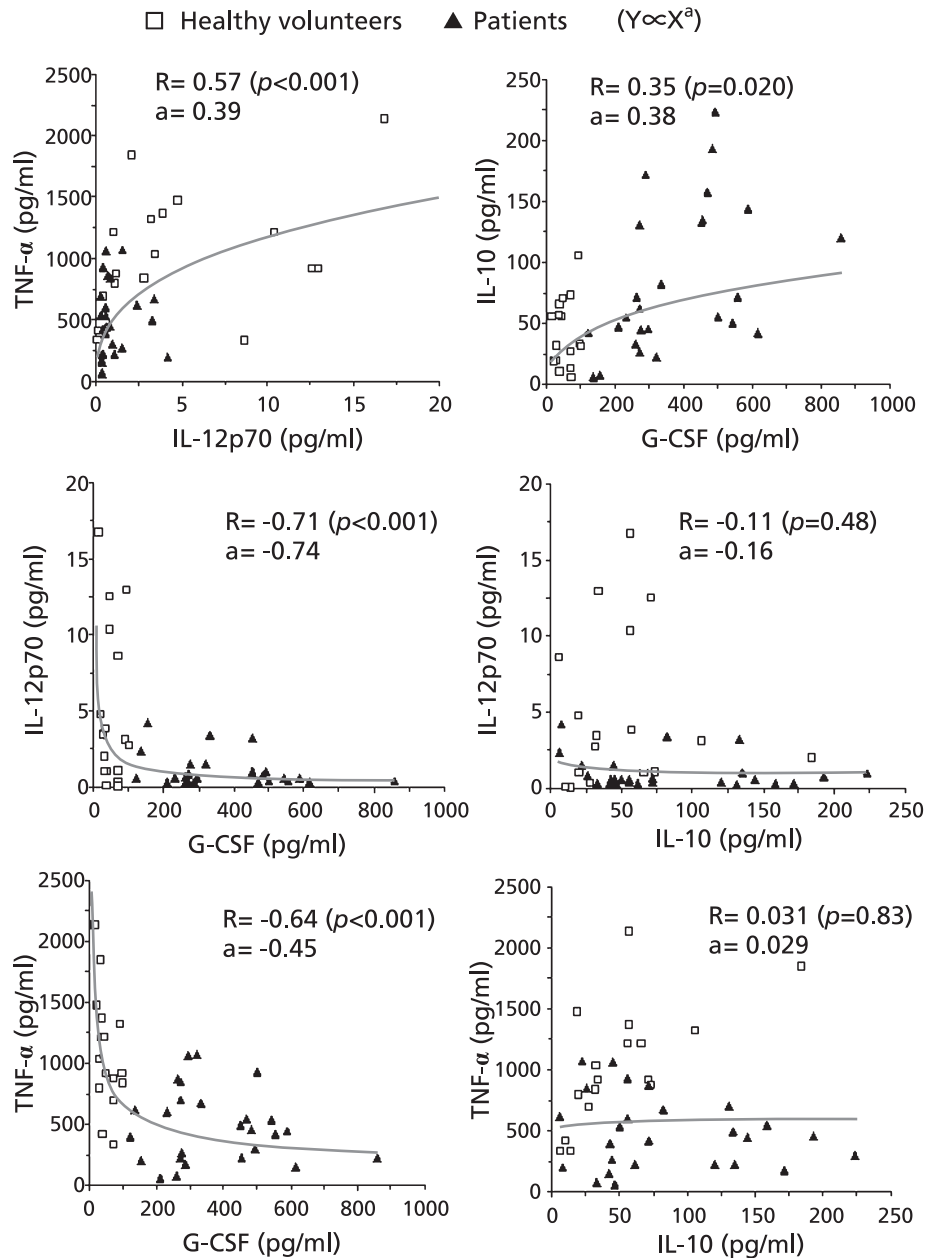


Fig. 3. Relationship between pro-inflammatory and anti-inflammatory cytokines secreted by LPS-stimulated leukocytes. Blood cells were collected from patients ($n = 26$, closed triangle) or age-matched healthy volunteers ($n = 18$, open square), and stimulated with $10 \mu\text{g/ml}$ LPS for 24 h. Correlations between pairs (X and Y) of the secreted cytokines (TNF- α , IL-12p70, G-CSF, IL-10) were analyzed. The degree of fit to a power-law distribution in which Y was proportional to X^a was calculated. Correlation coefficient (R) between $\log X$ and $\log Y$ and the slope (a) of the regression line ($(\log Y) / (\log X)$) are shown.

Fig. 5A, cAMP levels were significantly higher in patients ($p = 0.020$) compared to healthy volunteers. Fig. 5B shows the relationship between cAMP (X) and cytokine production (Y), fitted to exponential curves in which Y is proportional to e^{bX} , where e is the base of the natural logarithm and b is a constant. The levels of cAMP were inversely correlated with IL-12p70 production ($R = -0.81$, $p < 0.001$, $b = -0.13$) and directly correlated with G-CSF ($R = 0.72$, $p = 0.0020$, $b = 0.089$), MMP-9 ($R = 0.67$, $p = 0.0067$, $b = 0.041$) and IL-10 ($R = 0.54$, $p = 0.039$, $b = 0.049$) production. These results helped to explain why the relationship between cytokine production follows a power-law relationship. No statistically significant correlation was detected between cAMP and TNF- α levels in this experiment. In particular, the

production of IL-12p70 and G-CSF showed similar degrees of sensitivity, as expressed by the values of b , to intracellular cAMP, which explains the inverse relationship seen between IL-12p70 and G-CSF production seen in Fig. 3.

Discussion

LPS-stimulated monocytes from elderly humans have been reported to produce low amounts of cytokines, including TNF- α and G-CSF.⁽³⁰⁾ A significant association with age has also been reported for IL-12, but not IL-10, expression in feline monocytes.⁽³¹⁾ In this study, using whole blood cells from 47 healthy human volunteers, we have demonstrated that the LPS-induced

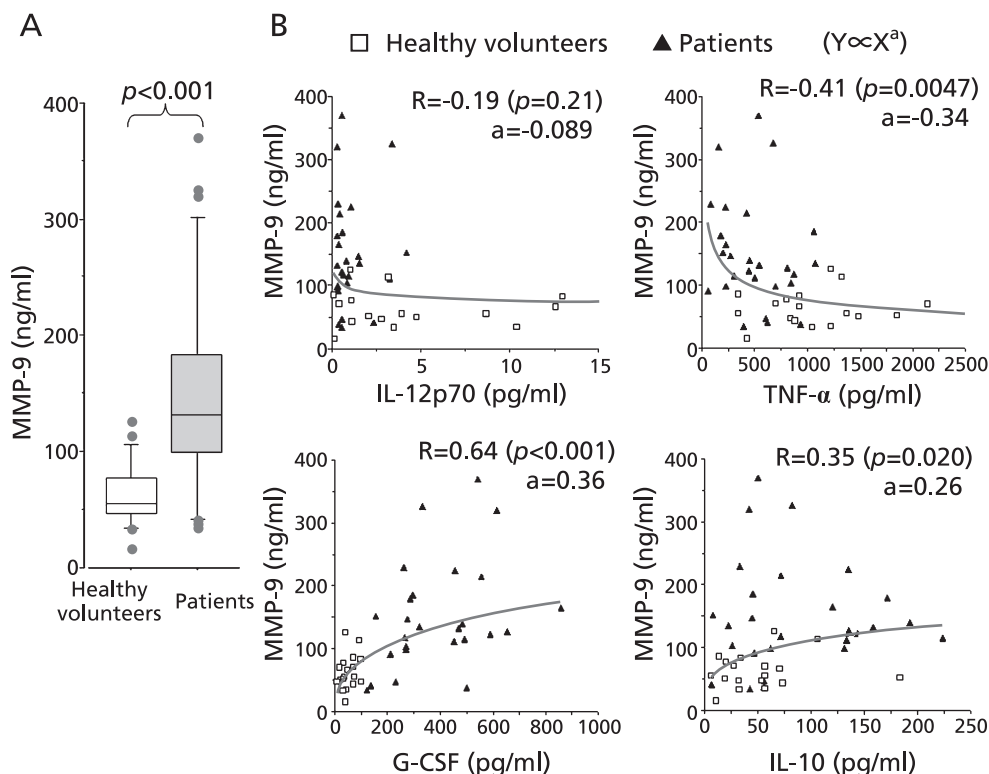


Fig. 4. Relationship between MMP-9 and cytokines secreted by LPS-stimulated leukocytes. (A) Blood from age-matched healthy volunteers ($n = 18$) and patients with gram negative bacterial pneumonia ($n = 26$) was incubated in the presence of $10 \mu\text{g/ml}$ LPS for 24 h and cytokine concentrations in the supernatants were compared statistically. (B) Concentrations of cytokines and of MMP-9 secreted by LPS-stimulated blood cells from patients ($n = 26$, closed triangle) or age-matched healthy volunteers ($n = 18$, open square) were plotted as X and Y. Correlation coefficients (R) were calculated by fitting the data to a power-law distribution in which Y is proportional to X^a .

secretion of TNF- α and IL-10 is reduced with age, although we did not detect any statistically significant changes in IL-12 or G-CSF expression. Furthermore, we found that most leukocytes from people showing high IL-12 production secreted very small amounts of G-CSF and vice versa.

Chen *et al.* reported that patients with acute bacterial infections who had low serum G-CSF concentrations on hospital admission were more likely to die.⁽³²⁾ Indeed maintaining high levels of G-CSF is known to be important for sustaining hematopoiesis in emergencies and for neutrophil differentiation, although it should be emphasized that G-CSF also has a regulatory role in controlling over-activation of host defense systems. TNF- α has an essential role in the pathogenesis associated with septic shock and in disseminated intravascular coagulation (DIC).⁽³³⁾ However, Weiss *et al.* reported that *in vitro*, the LPS-inducible secretion of TNF- α is downregulated and the secretion of G-CSF is upregulated in patients with septic shock.⁽³⁴⁾ Since G-CSF upregulation occurs earlier in the survivors than in the non-survivors, a rapidly elevated and sustained G-CSF response might contribute to the regulation of inflammation and so facilitate survival in endotoxin shock.⁽³⁰⁾ It is well known that G-CSF administration improves survival in animal models of sepsis and also endotoxin shock even when administered therapeutically after the onset of sepsis.⁽³⁵⁾

In terms of anti-inflammatory effects, IL-10, as well as G-CSF, is a candidate cytokine for inducing tolerance to alarm signals such as LPS. *In vitro* the inhibitory effects of IL-10 on the production of IL-12 and TNF- α are much greater than the effects of G-CSF, on a molar basis. Nevertheless, our study of gram negative bacterial pneumonia has clearly demonstrated that the production of IL-12 and TNF- α by the patients' leukocytes was inversely related to G-CSF production and not significantly related to IL-10

production. These results showed the significance of G-CSF in protecting hosts from tissue injury resulting from prolonged or repetitive alarm signals. G-CSF has been widely accepted to enhance the healing process in skin wounds^(36,37) as well as in cardiac infarction,⁽³⁸⁾ but there has been no evidence for a direct role for IL-10 in tissue repair. MMP-9 has been shown to have an active role in tissue repair in the liver.⁽³⁹⁾ In the lung, Choi *et al.* have reported a role for MMP-9 in tissue repair in cryptogenic organizing pneumonia.⁽⁴⁰⁾ In the more common interstitial pneumonia, pulmonary structure is extensively remodeled, whereas in cryptogenic organizing pneumonia architectural changes are minimal; levels of MMP-9 in broncho-alveolar lavage fluid are also higher in these patients. MMP-9 production was high in the patients recovering from pneumonia in our study and was more closely correlated with G-CSF production than IL-10 production.

The results of this study cannot in our view be adequately explained by a mild regulatory effect of secreted G-CSF *in vivo*. There is increasing evidence that intracellular cAMP levels determine cell functions such as patterns of cytokine production. For example, cAMP-elevating agents have been shown to enhance G-CSF promoter activity and attenuation of TNF- α production in a human monocytic cell line,⁽²⁶⁾ upregulate IL-10 mRNA expression in human peripheral blood mononuclear cells⁽²⁷⁾ and downregulate IL-12p40 mRNA levels in murine peritoneal macrophages.⁽²⁸⁾ Taken together, the upregulation of intracellular cAMP levels results in increased G-CSF and IL-10 expression and reduced IL-12 and TNF- α expression *in vitro*. Notably, recombinant G-CSF has been shown to increase intracellular cAMP levels in human PBMC in a dose-dependent fashion.⁽⁴¹⁾ These reports provide a molecular basis, supporting our *in vivo* results, for the production of G-CSF and proinflammatory cytokines (IL-12,

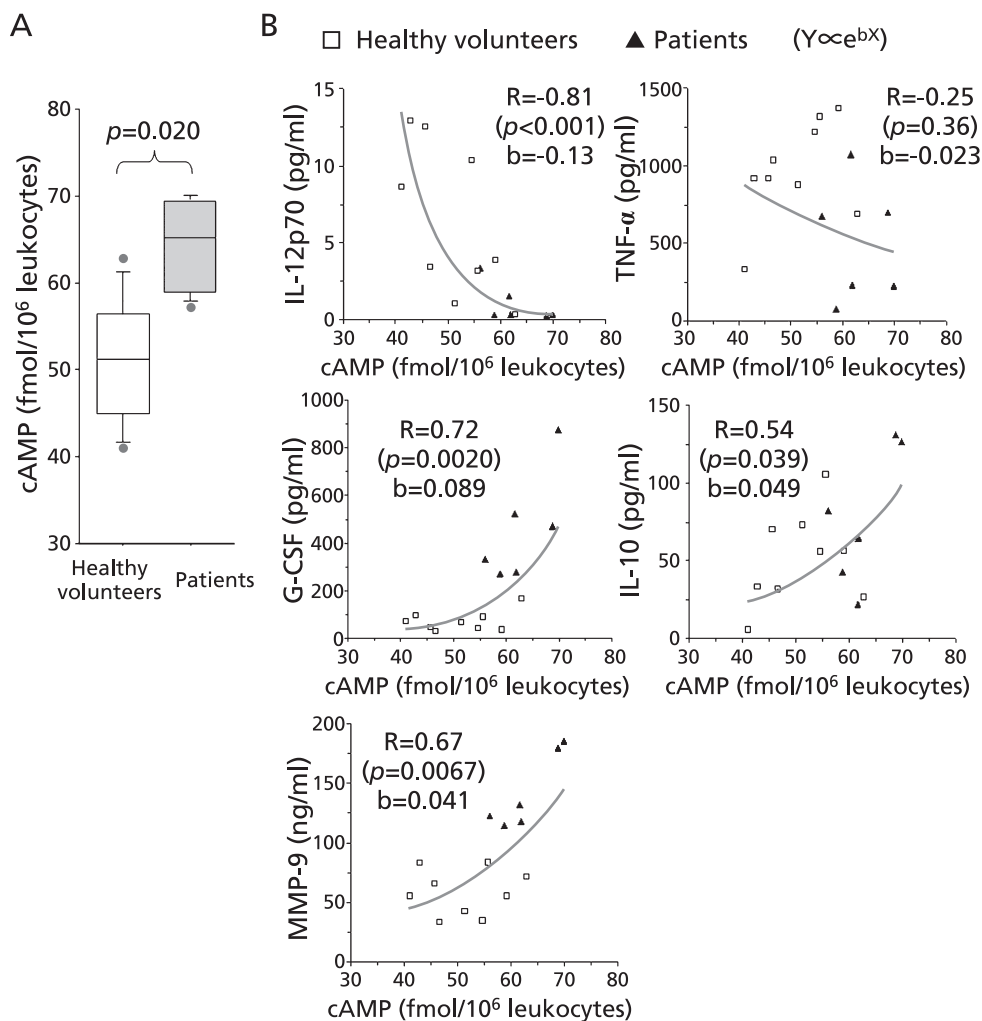


Fig. 5. Relationship between intracellular cAMP levels and cytokines secreted by LPS-stimulated leukocytes. (A) cAMP level in freshly isolated leukocytes from age-matched healthy volunteers ($n = 9$) and patients with gram negative bacterial pneumonia ($n = 6$) were compared statistically. (B) cAMP levels and the concentrations of cytokines and MMP-9 from blood cells stimulated with LPS *in vitro* were plotted as X and Y and fitted to an exponential distribution in which Y is proportional to e^{bX} . Correlation coefficients (R) between X and log Y and the slope of the regression line ((log Y)/X) (b) were calculated. Samples were from patients ($n = 6$, closed triangle) or age-matched healthy volunteers ($n = 9$, open square).

TNF- α) being inversely related in leukocytes, while also suggesting the importance of a positive feedback circuit in which secreted G-CSF enhances intracellular cAMP levels.

Chemical mediators synthesized during host inflammatory response, such as prostaglandin E2, histamine and the catecholamines, have been reported to increase intracellular cAMP levels and suppress IL-12 production in monocytes and monocyte-derived dendritic cells.⁽⁴²⁻⁴⁵⁾ In addition, catecholamines potentiate the LPS-induced expression of MMP-9 in human monocytes.⁽⁴⁶⁾ Thus the host response to repeated alarm signals such as LPS involves elevating cAMP levels and promoting both anti-inflammatory and tissue repair systems. This mechanism could

be a reasonable host adaptation for surviving severe acute inflammatory episodes, even though prolonged suppression of normal host responses might be expected to cause susceptibility to infection and carcinogenesis.

We have shown in this study that both G-CSF production by leukocytes stimulated with LPS *in vitro* and also the cAMP levels in freshly isolated leukocytes are useful indices of tolerance induction in patients who have survived gram negative bacterial pneumonia. From a clinical viewpoint, it is much more significant that we can predict the tolerant status of patients by simply measuring cAMP levels in leukocytes, as culturing cells with LPS is more time-consuming and costly.

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